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Abstract: Drought regimes can be characterized by the variability in the quantity of rainfall and the duration of rainless periods. However, most research on plant response to drought has ignored the impacts of rainfall variation, especially with regard to the influence of nonstructural carbohydrates (NSCs) in promoting drought resistance. To test the hypothesis that these components of drought differentially affect NSC dynamics and seedling resistance, we tracked NSC in plant tissues of tropical tree seedlings in response to manipulations of the volume and frequency of water applied. NSC concentrations decreased in woody tissues under infrequent-high watering but increased under no watering. A faster decline of growth relative to stomatal conductance in the no watering treatment was consistent with NSC accumulation as a result of an uncoupling of growth and photosynthesis, while usage of stored NSCs in woody tissues to maintain function may account for the NSC decline under infrequent-high watering. NSCs, and specifically stem NSCs, contributed to drought resistance under severe water deficits, while NSCs had a less clear role in drought resistance to variability in water availability. The contrasting response of NSCs to water variability and deficit indicates that unique processes support seedling resistance to these components of drought.

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Contrasting non-structural carbohydrate dynamics of tropical tree seedlings under water deficit and variability

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Summary

1. Drought regimes can be characterized by the variability in the quantity of rainfall and the duration of rainless periods. However, most research on plant response to drought has ignored the impacts of rainfall variation, especially with regard to the influence of non-structural carbohydrates (NSC) in promoting drought resistance.
2. To test the hypothesis that these components of drought differentially affect NSC dynamics and seedling resistance, we tracked NSC in plant tissues of tropical tree seedlings in response to manipulations of the volume and frequency of water applied.
3. NSC concentrations decreased in woody tissues under infrequent-high watering but increased under no watering. A faster decline of growth relative to stomatal conductance in the no watering treatment was consistent with NSC accumulation due to an uncoupling of growth and photosynthesis, while usage of stored NSC in woody tissues to maintain function may account for the NSC decline under infrequent-high watering.
4. NSC, and specifically stem NSC, contributed to drought resistance under severe water deficits, while NSC had a less clear role in drought resistance to variability in water availability. The contrasting response of NSC to water variability and deficit indicates unique processes support seedling resistance to these components of drought.

Keywords

Climate change; Drought resistance; Plant-climate interactions; Relative growth response; Stomatal conductance; Tropical forests

Introduction

42 The importance of drought in shaping distributions of tree species in forests across the
43 globe is increasing as climate change progressively alters the quantity (i.e. total rainfall),
44 frequency (i.e. daily, weekly and monthly patterns) and intensity (i.e. rate of rainfall during
45 events) of rainfall (Timmermann *et al.*, 1999; Huntington, 2006; Hartmann, 2011; Lewis *et al.*,
46 2011; Beier *et al.*, 2012). This increase in the role of drought on community dynamics and forest
47 mortality has generated an increased interest in understanding traits that promote drought
48 resistance. Non-structural-carbohydrate concentration (NSC) is one such trait that has been
49 shown to be important for prolonging drought survival during severe water deficits (O'Brien *et*
50 *al.*, 2014). Research on the role and response of NSCs to drought has commonly focused on
51 severe deficits leading to mortality (Anderegg *et al.*, 2012; Adams *et al.*, 2013; Hartmann *et al.*,
52 2013; O'Brien *et al.*, 2014). However, the importance of NSC concentration for the response of
53 trees to long-term minor deficits and variation in rainfall frequency has largely been ignored.

54 Plant response to drought is commonly categorized along a spectrum from isohydric (i.e.
55 strong stomatal control with decreasing water availability) to anisohydric (i.e. weak stomatal
56 control) (McDowell *et al.*, 2008; Mitchell *et al.*, 2013). An isohydric strategy can lead to
57 mortality by carbon starvation if photosynthesis is inhibited and NSC reserves are depleted
58 (McDowell *et al.*, 2008; Sala *et al.*, 2012). Anisohydric species that maintain growth and
59 stomatal conductance during severe water deficits risk mortality from hydraulic failure and
60 desiccation or carbon starvation due to water limitation of NSC transport (McDowell *et al.*,
61 2008; Bartlett *et al.*, 2012; Sala *et al.*, 2012; Hartmann *et al.*, 2013). The effect of fluctuating
62 water supply on NSC dynamics is less understood, as shifts from wet to dry soil conditions may
63 induce a different response to that of water deficit alone (Parent *et al.*, 2008). We hypothesize
64 three potential responses of NSC stores to variable water conditions: 1) transient decreases in

NSC concentrations due to use of stored NSC for maintenance of metabolic function and growth during dry periods, 2) transient decreases in NSC concentrations due to usage for rapid recovery of growth following dry periods or 3) transient increases in NSC concentrations due to an uncoupling of growth and photosynthesis during dry periods.

Furthermore, these different drought regimes may alter relative abundance among species across the isohydric – anisohydric spectrum. For example, species that maintain growth and risk hydraulic failure (i.e. anisohydric) may have an advantage under variable water conditions – which never reach the threshold of hydraulic failure before a rainfall event occurs – because continued growth will provide them with a size advantage after continuous rainfall returns. Conversely, long-term water deficits may favour species that maintain high water potentials (i.e. isohydric) at the cost of slow growth under high water availability. However, the benefit of slower growth may be a lower risk of mortality if these species maintain hydraulic function when soil water potential drops below the wilting point during severe drought events (Bartlett *et al.*, 2012; Hartmann *et al.*, 2013). Therefore, these different aspects of drought may have differential effects on relative abundance of species under variable rainfall versus severe drought events.

Aseasonal tropical forests provide an appropriate system to test the effects of rainfall variability and water deficits as both elements vary across a range of temporal scales. The rainfall regime of aseasonal tropical forest ecosystems is characterized by alternating short rainless periods and high or extreme rainfall, punctuated by irregular supra-annual droughts associated with El Niño Southern Oscillation events (Walsh & Newbery, 1999; Engelbrecht & Kursar, 2003; Engelbrecht *et al.*, 2007; Beier *et al.*, 2012). Previous work on the impacts of moisture variability in aseasonal forests has largely focused on landscape level mortality rates due to severe drought (Nakagawa *et al.*, 2000; Gibbons & Newbery, 2002; Potts, 2003; Slik,

2004; Itoh *et al.*, 2012), but less has been done to examine the growth and physiological sensitivity of species to water deficits and variability and the traits that promote the ability of a species to survive and maintain function during drought (Burslem *et al.*, 1996; Tyree *et al.*, 1998; Cao, 2000; Baltzer *et al.*, 2008). Recent research has shown that higher NSC concentrations in tropical tree seedlings prior to drought prolong survival during drought (O'Brien *et al.*, 2014), but studying NSC concentration during drought remains necessary to elucidate the role of fluctuations of NSC throughout various plant tissues in explaining seedling response.

We monitored NSC changes for seedlings of 10 Bornean tree species (Table 1 and S1) during three different drought treatments relative to a frequently and well-watered control: 1) minor water deficit (frequent-low volume: the frequency of application was every two days, the same rate as the control, but the volume of water was reduced to about 20% of the control volume), 2) variable water (infrequent-high volume: volume same as the control but applied every 15 days) and 3) severe deficit (no watering). We measured NSC concentrations in leaf, stem and root tissues through time for each drought treatment and examined relationships between changes in relative growth rates across species, xylem water potential responses and NSC storage or consumption in order to identify important NSC variables associated with resistance to the different drought treatments.

Materials and methods

Study site

We conducted this experiment at the Malua Field Station (N05º05'20'' E117º38'32''; 102 MASL), which is located approximately 22 km north of Danum Valley Field Centre in the Malaysian state of Sabah (Hector *et al.*, 2011). Mean annual rainfall (s.e.m.) recorded in Danum

Valley from 1986-2010 was 2848.5 (94.0) mm, and over the last 100 years, rainfall has increased in variability and severity of water deficits (Walsh & Newbery, 1999; Fig. S1). The experiment was conducted in 15 shade-houses under two layers of 70% shade-cloth. Below the shade-cloth clear plastic polyethylene sheeting was used to exclude rainfall. The shade-houses provided the seedlings with an average (s.e.m.) of 3.9% (0.1) of full daylight photosynthetically active radiation (PAR) and a red:far-red ratio of 1.07 (0.01). Light was measured by simultaneous shade-house and open sky PAR sensors (SKP 210 quantum sensor; Skye instruments LTD, Llandrindod Wells, Powys, UK). Temperature and humidity in the shade-houses were similar to that of measurements made at the weather station at the Malua Field Station (Methods S1).

Seedlings

Seeds from ten species of shade-tolerant forest trees were collected during a landscape scale masting event in August 2010 (Table 1; Table S1). The species selected include eight species from the family Dipterocarpaceae, one from the Fabaceae and one from the Bombacaceae. We placed seeds under wet burlap sacks to germinate. After germination, each seed was planted into large pots (20 cm diameter x 36 cm height; 11 L volume) filled with homogenized forest soil within the shade-houses. Seedlings were watered every two days for three months until all seedlings had abscised their cotyledons. We replaced individuals that died during the first two months from nursery grown seedlings that had been collected during the same masting event.

Experimental design

On 19 November 2010, we assigned at random a selection of 22 seedlings of each species to each of four treatments (Fig. S2): 1) 240 mm of water per month distributed in equal amounts

every two days (frequent-high volume; control), 2) 50 mm of water per month distributed in equal amounts every two days (frequent-low volume), 3) 240 mm of water per month distributed in equal amounts every 15 days (infrequent-high volume) and 4) complete dry down (no water).

Seedling growth, biomass and leaf area

We measured the height, diameter and leaf area of every seedling prior to the start of the treatments and every month thereafter. Leaf area was estimated from measurements of the length and width of every leaf on each seedling. These measures were used to fit linear models of leaf area from destructive harvests of seedlings (mean R^2 for all species = 0.93, range = 0.86 – 0.97; Fig. S3). Loss of leaf area from herbivory or browning was estimated visually as the percentage of the whole leaf lost in 5% increments.

We destructively harvested a sample of seedlings prior to the start of the treatments and then approximately 30, 60 and 120 days after the treatments began. A final harvest of all living seedlings occurred 150 days after the treatments began. We harvested one seedling of each species in each treatment at pre-dawn (03:00-06:00), morning (08:00-10:00), mid-day (12:30-15:00) and late-afternoon (17:00-19:00) and made a variety of trait measurements on each individual. In order to assess the water status of each seedling, we measured leaf water potential on a single leaf (or a cluster of leaves for small seedlings when petioles were too small to fit into the chamber) and stem water potential on a single segment of the stem (~8 cm in length) using a Scholander pressure chamber (model 670, PMS Instrument Co., Corvallis, Oregon, USA). We took photographs of all the leaves and analyzed their leaf area using ImageJ software (Rasband, 2013). These measured leaf areas were used to generate the linear models for estimating leaf area from the length and width measurements (Fig. S3). We removed all soil and measured longest

154 root length. Seedlings were dried at 64° C to a constant weight, and we weighed leaves, stem and
155 roots separately to assess biomass allocation.

156 *Seedling monitoring*

157 We monitored the seedlings every two days for changes in leaf morphology. We recorded
158 the date of leaf browning, decline in leaf angle, initial leaf loss and total leaf loss. Approximately
159 every ten days, we made measures of stomatal conductance (g_s) from 08:00 until 18:00 with a
160 steady state diffusion porometer (model SC-1, Decagon Devices, Inc., Pullman, WA, USA).
161 During each daily course, we measured 120 seedlings (three individuals of each species in each
162 treatment) every three hours. Each of the 120 seedlings received three stomatal conductance
163 measurements per daily course (morning, mid-day and afternoon). Mid-day stomatal
164 conductance through time was used to assess the effect of treatments on stomatal closure.

165 *Environmental conditions*

166 In order to measure temperature differences among treatments, we placed Thermocron
167 Ibuttons (model DS1921G-F5#, Maxim Integrated Products, Inc., Sunnyvale, CA, USA) in all
168 four treatments in each shade-house. In order to capture air and soil temperature differences,
169 Ibuttons were buried 5 cm within the soil and suspended on a stick 5 cm above the soil and set to
170 a 30 minute measurement interval. Volumetric soil moisture content at the top and bottom of the
171 pot was measured on 1-2 seedlings of each species in each treatment every week with an ML2x
172 Theta Probe and HH2 moisture meter (Delta-T Devices, Burwell, Cambridge, UK). The
173 relationship between soil water potential and volumetric soil moisture content was determined
174 using the filter paper method (Deka *et al.*, 1995; O'Brien *et al.*, 2013). We measured relative
175 humidity near the shade-houses every 30 minutes throughout the course of the experiment with a

digital humidity probe (SKH 2000 probe, Skye instruments LTD, Llandrindod Wells, Powys, UK).

Non-structural carbohydrate analysis

We used three to four seedlings of each species from the first harvest, one seedling of each species in each treatment for each intermediate harvest and three to four seedlings of each species in each treatment from the last harvest to quantify non-structural carbohydrate (NSC) concentrations in the leaf, stem and root. Seedlings of three species did not survive until the final harvest therefore the last seedlings to die were used for NSC analysis. At harvest, seedlings were immediately microwaved to stop enzymatic activity. We ground tissue samples with a ball mill and used 15-16 mg of sample for NSC analysis. We extracted soluble sugars with 80% ethanol at 27° C for one night followed by two additional 2 hour periods (Marquis *et al.*, 1997; Myers & Kitajima, 2007). We digested the remaining starch with amyloglucosidase (A-7420, Sigma-Aldrich, Co., St. Louis, MO, USA). The concentrations (mg per mg) of simple sugars and starch (measured as glucose-equivalents) were measured at 487 nm by spectrophotometry after a 30 minute phenol-sulphuric acid reaction (Dubois *et al.*, 1956; Ashwell, 1966). We used a weighted NSC concentration for total NSC, starch and soluble-sugars in a seedling to account for differences among seedlings in tissue biomass (i.e. each tissue concentration was multiplied by its dry biomass and their sum was divided by the whole seedling dry biomass).

Analysis

In order to assess the amount of soil drying and re-wetting in the infrequent-high watering treatment, we modeled soil water potential in this treatment as a function of time (a continuous variable, in days) interacting with a variable for soil water potential before and after

198 watering (a fixed factor with 2 levels, before and after water addition) using a linear mixed-
199 effects model with a random intercept for individual seedling. The absolute value of soil water
200 potential was log-transformed to meet assumptions of linearity, and the results are presented on
201 the transformed scale; therefore, a steeper positive slope indicates faster soil drying. We used a
202 linear mixed-effects model to assess declines in soil water potential in the no watering treatment
203 as a function of time since the start of the treatment (a continuous variable, in days) and species
204 identity (a fixed factor with 10 levels) with a random intercept for individual seedling. Again, the
205 absolute value of soil water potential was log-transformed to meet assumptions of linearity. We
206 analyzed soil temperature as a function of treatment (a fixed factor with 4 levels) using linear
207 mixed-effects models with random intercepts for hour of the day, date and individual seedling.

208 We assessed seedling response to drought treatments by analyzing living biomass (dead
209 material was given a biomass of zero to account for the negative effect of dieback and mortality)
210 as a function of time since the start of the experiment (a continuous variable), watering treatment
211 (a fixed factor with 4 levels), species identity (a fixed factor with 10 levels) and all interactions
212 (including the three-way interaction between treatment, species and time) with a generalized
213 least squares model. We used initial leaf area for each individual seedling as a covariate to
214 account for differences in initial seedling size, which was necessary to account for differences in
215 water use (Fig. S4). The results of this analysis allowed us to calculate a relative growth rate of
216 biomass (RGR) for each species in each treatment using an initial standardized seedling size (125
217 cm^2). In order to assess the effect of watering treatments on drought response among species, we
218 calculated the difference in mean RGR between a treatment and the control treatment for each
219 species as an assessment of drought resistance (ΔRGR). Therefore, a negative ΔRGR value for a
220 species in a drought treatment indicates that the treatment reduced mean RGR relative to the

221 control – due to either slower growth, decreasing size or dieback/mortality (i.e. zero living
222 biomass).

223 We analyzed the response of NSC concentration as a function of time since the start of
224 the experiment (a fixed factor with 5 levels, one for each harvest), watering treatment (a fixed
225 factor with 4 levels) and their interaction. A random intercept for species identity was used in the
226 model (a random factor with 10 levels). Furthermore, in order to examine the changes in NSC
227 within seedlings, we analyzed NSC concentration in leaf, stem and root tissues and for starch and
228 soluble-sugar separately using the same model as above (Fig. S5). Additionally, we calculated
229 the difference in woody tissue (average allocation to stem and root, ΔWoody) and leaf tissue
230 (ΔLeaf) between the initial and all other time-points for NSC, soluble-sugars and starch
231 concentrations. In order to determine the effects of watering treatments on changes in allocation,
232 we analyzed ΔWoody and ΔLeaf tissue concentrations as a function of treatment (a fixed factor
233 with 4 levels). We plotted these metrics against each other for NSC, soluble-sugars and starch
234 concentrations and tested the difference in allocation to source (leaf tissue) and sink tissues
235 (woody tissues) under each treatment relative to the initial allocation.

236 We used Pearson correlations between ΔRGR across species in each drought treatment
237 and species traits in the control treatment to determine the traits that improved drought
238 resistance. We used traits from the control treatment because the relationship between baseline
239 traits and drought response was of interest and not the effect of drought on traits. The traits
240 assessed in the correlation analysis were average initial biomass, RGR in the control treatment,
241 total seedling and leaf, stem and root NSC concentrations and total seedling and leaf, stem and
242 root starch and soluble-sugar concentrations. A metric for xylem sensitivity to decreasing soil
243 moisture potential – calculated as the mean difference in stem water potential and soil water

potential in the no watering treatment at the last two harvests ($\Psi_x - \Psi_s$) – was used to assess the role of xylem tolerance to soil drying on drought resistance. We also used ΔWoody tissue soluble-sugar concentration in order to assess the role of variation in osmotically important components for drought resistance. Significant correlations between ΔRGR and baseline traits were analyzed with generalized least squares models to assess the effect of baseline traits on drought resistance. Standardized major axis regression (which accounts for variation in both variables) was used to visualize relationships between correlated traits (Warton *et al.*, 2006).

The effect of treatments on soil water potential and NSC concentration were analyzed with the lme function in the nlme package (Pinheiro & Bates, 2000) of the R statistical software version 3.0.2 (R Development Core Team, 2013). Generalized least squares analysis on ΔRGR and traits was performed with the gls function in the nlme package (Pinheiro & Bates, 2000). Correlations were performed with the rcorr function in the Hmisc library. Standardized major axis regression between traits was performed with the sma function in the smatr package (Warton *et al.*, 2012). Analysis of soil temperature were performed with the lmer function in the lme4 library because of the complexity of the random effects (Bates & Maechler, 2011), and we calculated the 2.5 and 97.5 percent quantiles from 1000 resamples of the parameter estimates using the sim function in the arm library in order to get 95% confidence intervals (CIs) around temperature estimates because the lme4 library does not produce CIs (Gelman & Hill, 2007; Bagchi *et al.*, 2011).

Results

Soil drying

There were no differences in soil temperature between treatments (control = 25.5° C, 95% CI: 25.0 – 25.9, frequent-low = 25.4° C, 25.0 – 25.7, infrequent-high = 25.6° C, 25.2 – 26.0 and no water = 25.5° C, 25.1 – 25.9). Soil water potential remained high in the control, frequent-low and infrequent-high watering treatments (Fig. 1a-c). However, pre-watering soil water potential in the infrequent-high watering treatment declined with days since the start of the treatment (slope of natural-log MPa with days = 0.004, 95% CI: 0.003 – 0.005) indicating that soil water was depleted more rapidly as plants grew larger. Post-watering soil water potential in the infrequent-high watering treatment remained relatively constant through time (slope of natural-log MPa with days = -0.0002, 95% CI: -0.002 – 0.002), which implies that every watering event was recharging the soil water to an equivalent extent. Soil water potential began to decline significantly from zero in the no watering treatment after approximately 45 days (Fig. 1d). This decline varied slightly among species with *D. oxleyanus* having the steepest change (slope of natural-log MPa with days = 0.07, 95% CI: 0.04 – 0.09) and *H. nervosa* having the slowest change (slope of natural-log MPa with days = 0.04, 95% CI: 0.03 – 0.05; Fig. 2a).

Seedling mortality, growth and stomatal response

Mortality was not significantly different among the control, frequent-low and infrequent-high watering treatments (control = 2.3%, 95% CI: 0.2 – 4.3, frequent-low = 2.3%, -95% CI: 0.5 – 5.1 and infrequent-high = 1.4%, 95% CI: 0.0 – 2.8) but was significantly higher in the no watering treatment (29.5%, 95% CI: 18.8 – 40.3). All species exhibited higher mortality in no watering than the control, frequent-low and infrequent-high watering treatments (Table S2).

The timing and extent of leaf loss varied among species with *K. excelsa* dropping leaves first and *H. nervosa* last in response to no water. During the 150 days of no water, only three

287 species had a positive RGR (*H. nervosa*, *K. excelsa* and *S. parvifolia*), and only *H. nervosa* had
288 an RGR statistically indistinguishable from the control. *S. macrophylla* had the fastest decline in
289 living biomass (-0.15 g g^{-1} day^{-1}, 95% CI: -0.23 – -0.1, Fig. 2b). Because large seedlings declined
290 faster due to earlier soil drying (i.e. due to higher water demands), we also calculated decline in
291 soil water potential with time for each species in the no watering treatment and modelled RGR as
292 a function of soil water potential instead of days. Decline in RGR among species with decreasing
293 soil water potential was significantly correlated with decline in RGR with days (Pearson r = 0.95,
294 95% CI: 0.8 – 0.99). Since these two metrics were highly correlated, we retained the use of RGR
295 as a function of days in order to maintain comparisons across treatments instead of using RGR as
296 a function of soil water potential solely for no watering.

297 *D. lanceolata*, *S. argentifolia* and *S. beccariana* had significantly lower RGR in the
298 infrequent-high watering treatment relative to the control while all other species had RGRs that
299 were statistically indistinguishable from the control (Fig. 2b). *D. lanceolata* and *S. beccariana*
300 were the only species which grew significantly less in the frequent-low watering treatment
301 relative to the control (Fig. 2b).

302 Stomatal conductance was statistically indistinguishable among all treatments for 60 days
303 (Fig. S6). After 80 days stomatal conductance in the no watering treatment was statistically
304 lower than the other three treatments (stomatal conductance difference between no watering and
305 other treatments = -166 mmol m^{-2} sec^{-1}, -299 – -33). In this treatment, mean stomatal
306 conductance was significantly below 100 mmol m^{-2} sec^{-1} after 100 days and reached a minimum
307 after 150 days (~10 mmol m^{-2} sec^{-1}). The other three treatments were never statistically
308 distinguishable from each other for the entire experiment. Species varied in their decline in

309 stomatal conductance with *P. malaanonan* dropping significantly below 100 mmol m^{-2} sec^{-1} after
310 100 days while *S. macrophylla* took only 44 days (Fig. 2c).

311 *Non-structural carbohydrate response*

312 Non-structural carbohydrate concentrations in seedlings varied with drought treatment:
313 no watering caused a significant increase in NSC above the control while seedlings exposed to
314 infrequent-high watering had significantly lower NSC concentrations than those in the control
315 (Fig. 3a). In the no watering treatment, leaf NSC concentration increased above that of the
316 control for 30 days (difference in leaf NSC in no watering and control = 3.2%, 95% CI: 0.7 –
317 5.6, Fig. 3b) and then decreased to values similar to that of the other treatments. Stem NSC
318 concentration in the no watering treatment increased over the 150 days to concentrations
319 significantly higher than the control (mean difference in stem NSC between no watering and
320 control = 3.8%, 1.5 – 6.0, Fig. 3c) while root NSC remained higher in the no watering treatment
321 but fluctuated more reaching values significantly higher than the controls after 30 days (4.0%,
322 1.4 – 6.7) and 150 days (3.6%, 1.1 – 6.1, Fig. 3d). Soluble-sugars remained relatively constant
323 trough time in the leaf, stem and root while starch concentrations increased in the stem and root
324 (Fig. S5g,k).

325 NSC concentration in the infrequent-high watering treatment contrasted that of no
326 watering with a decline to minimum values around 60 days (mean difference in stem NSC
327 between infrequent-high watering and control = -2.0%, -3.7 – -0.4) followed by a recovery to a
328 concentration similar to the control (Fig. 3a). Stem NSC was the main driver of this decline as it
329 reached a minimum at 60 days (Fig. 3b). This decline was evident in both starch and soluble-
330 sugar concentrations, but the recovery was delayed in soluble-sugars (Fig. S5e – l). NSC

concentrations in the frequent-low watering treatment were statistically indistinguishable from the control throughout the experiment.

Under no watering, woody tissue significantly increased in total NSC concentration (2.3%, 95% CI: 1 – 3.5), which was driven by a significant increase in starch (2.4%, 1.2 – 3.5, Fig. 4a,b). Soluble-sugars in woody tissue on average were not significantly different from the initial time-point under no watering (Fig. 4c). Under infrequent-high watering, woody tissue significantly decreased in total NSC (-1.0%, -2.0 – -0.1, Fig. 4a) and soluble-sugar (-1.3%, -1.8 – -0.7, Fig. 4c) concentrations while on average starch concentration remained similar to the initial time-point. Furthermore, soluble-sugars in woody tissues were significantly lower under infrequent-high watering than the controls (difference between infrequent-high and control watering = 0.5%, 0.1 – 0.8). Allocation under control and frequent-low water were similar for all NSC components and only varied significantly from the initial time-point with a decrease in soluble-sugars (-0.8%, -1.3 – -0.3, Fig. 4c). Allocation to leaf tissue was statistically indistinguishable from the initial time-point for all treatments (Fig. 4).

Baseline traits and drought resistance

In the infrequent-high watering treatment, ΔRGR was positively correlated with ΔWoody soluble-sugars (r = 0.74, 95% CI: 0.2 – 0.9), which indicates that species with a greater negative biomass response to infrequent-high watering also had a larger reduction in soluble-sugar concentrations in their woody tissues. In the frequent-low watering treatment, ΔRGR declined with increasing initial stem NSC (slope of stem NSC to ΔRGR for frequent-low = -0.005, 95% CI: -0.01 – -0.002). Initial size negatively affected ΔRGR in the no watering treatment (slope of ΔRGR with increasing biomass = -0.03, -0033 – -0.02, Fig. 5a) whereby larger seedlings had

353 more negative ΔRGR. Initial size did not significantly affect the other two drought treatments
354 (Table S3). Fast growth in the control negatively affected ΔRGR (slope of ΔRGR with
355 increasing RGR in the control = -3.1, -5.2 – -1.0, Fig. 5b). ΔRGR in the no watering treatment
356 significantly increased with NSC concentration (slope of ΔRGR with increasing NSC = 0.03,
357 0.01 – 0.04, Fig. 5c) and stem NSC concentration (slope of ΔRGR with increasing stem NSC =
358 0.03, -0.00007 – 0.1, Fig. 5d). Initial biomass and RGR in the control were highly correlated (r =
359 0.7, 0.1 – -0.9, Fig. 6a). However, these variables traded off with weighted NSC concentration
360 (biomass-NSC r = -0.8, -0.9 – -0.3 and RGR-NSC r = -0.9, -0.97 – -0.6, respectively, Fig. 6b,c).
361 RGR in the control treatment also traded off with stem NSC concentration (r = -0.7, -0.9 – -0.03,
362 Fig. 6d, Table S4).

363 **Discussion**

364 We examined the effect of multiple components of the drought regime on NSC storage
365 and depletion, and the importance of NSC storage in mediating drought response of tropical tree
366 seedlings. Interestingly, infrequent-high watering (alternation between sufficient water and
367 deficit) and no watering (sustained and increasing water deficit) were associated with contrasting
368 patterns of NSC concentration in woody tissues, resulting in accumulation of NSC under
369 sustained water deficit and consumption of NSC under a variable water regime. These NSC
370 responses were coupled with contrasting growth responses to drought which suggests that
371 accumulation occurred in response to inhibited growth under no water and depletion occurred
372 due to the maintenance of growth, especially in the first 120 days, under infrequent-high water.
373 NSC traits were not strong predictors of seedling growth under frequent-low and infrequent-high
374 watering. However, under prolonged severe drought with no water, species with greater total and
375 stem NSC concentrations, slower growth and smaller initial size were less susceptible to

reduction in growth during drought. These results suggest that NSC storage trades off with growth rate when water availability is non-limiting and that baseline NSC storage is indicative of the relative drought resistance among species.

Differential NSC response to infrequent-high water and no water

The contrasting effects of infrequent-high and no water on NSC concentration indicate differential seedling responses to the two treatments: NSC consumption and continued growth under infrequent-high water and NSC accumulation and inhibited growth under no water. Usage of NSC stores in the stem and roots to maintain metabolic function and sustain growth may account for the decrease in NSC concentrations during the first 120 days in the infrequent-high watering treatment. Alternatively, NSC use for growth following watering events may also cause the decrease in NSC concentration in woody tissues, but measurements of NSC concentrations pre- and post-watering would be necessary to distinguish between these two mechanisms. Not surprisingly, the strongest declines occur in osmotically active soluble-sugars, which may support the hypothesis that NSC is being used during short dry-periods to maintain function. However, there was no evidence for translocation of NSC from source to sink tissues as leaf tissue concentration never deviated significantly from initial concentrations.

In the infrequent-high watering treatment NSC concentrations recovered to values that were similar to the control after 150 days. We hypothesize this was due to an increase in water deficit between watering events as seedlings grew larger and water demands increased causing growth to slow or stop and NSCs to accumulate. This hypothesis is supported by the significant decrease in pre-watering soil moisture availability through time in the infrequent-high watering treatment. Alternatively, an adaptation of seedlings to infrequent-high watering with time by

increased allocation to root growth would reduce the effect of the dry periods and may explain the NSC recovery. Therefore, species could avoid the dry period and recover their NSC concentrations (Poorter & Markesteijn, 2008; Markesteijn & Poorter, 2009). Our results cannot directly elucidate the cause of this recovery in NSC concentrations, but there is no support for adaptation of morphology because we did not find significant changes to the root-mass fraction or the root mass to leaf area ratio in the infrequent-high watering treatment (Fig. S6). Furthermore, the trajectory of change in the stem and root NSC concentrations indicates a steady increase from 60 to 150 days (Fig. 3c,d), and if NSC concentrations continued to increase, then initial NSC accumulation from uncoupled growth and photosynthesis can be assumed.

The increase in NSC concentrations in the no watering treatment is consistent with an uncoupling of growth and photosynthesis (Muller *et al.*, 2011). Growth began to decline significantly around 30 days of no water (Fig. S4) while stomatal closure did not decline significantly until 45 days of no watering for a few species and more than 70 days for most species (Fig. 2c) while sustained low conductance (<100 mmol m^{-2} sec^{-1}) was not achieved until 80 days of no watering (Fig. S6). Therefore, this decline in growth likely occurred before photosynthesis was inhibited resulting in an accumulation of NSC (Muller *et al.*, 2011). Our results support other studies that have found an accumulation of stored NSC in fast-growing species (Mitchell *et al.*, 2013). However, a dry-down experiment in pots has its limitations as it represents a simplified drought scenario. It is possible that soil drying could occur more rapidly during a natural drought than in our experiment due to reduced cloud cover, more competition for soil water with overstorey trees and higher vapour pressure deficits. Research examining plant response during severe El Niño driven droughts is the next step to validate our results in a natural setting.

421 Interestingly, leaf NSC concentrations behaved similarly in all treatments (remaining
422 statistically indistinguishable among treatments and across time) although an initial increase in
423 the no watering treatment for 30 days was observed. Furthermore, starch increased more than
424 soluble-sugars under no watering (Fig. S5g,k) while under infrequent-high watering only
425 soluble-sugars decreased. This result may indicate that soluble-sugars are important for short-
426 term stress tolerance, while accumulation under severe water shortage promotes starch storage.

427 *Traits promoting drought resistance*

428 Resistance to severe water deficits from no watering was associated with the multiple
429 interrelated variables of initial size, baseline RGR, total weighted NSC and stem NSC. The
430 relationship between ΔRGR and initial biomass was driven by the three largest species (Fig. 5c).
431 Although these three species experienced dieback earlier than the other species because of more
432 depletion of soil water, mortality of the two species with the largest seedlings (*S. macrophylla*
433 and *D. oxleyanus*) also occurred at higher soil water potentials than other species (Fig. 2a).
434 Therefore, these larger seedlings depleted the soil water faster but were also less tolerant of low
435 soil water potentials than seedlings of other species. Size artifacts alone did not account for
436 differences in ΔRGR in the no watering treatment. Initial leaf area displayed a trade-off with
437 total weighted NSC concentration, which indicates that the larger individuals stored relatively
438 less NSC (Fig. 6a). Furthermore, seed size correlated with larger initial leaf area (0.98, 0.90 –
439 0.99), and seedlings of large seeded species grow faster in the first few months of development
440 (O'Brien *et al.*, 2013). Therefore, species with faster pre-drought growth rate had lower NSC
441 stores relative to their size prior to the start of the drought, which may have caused the rapid
442 subsequent declines in ΔRGR and higher mortality in response to drought (O'Brien *et al.*, 2014).

443 Species with greater baseline NSC concentration prior to drought were less affected by
444 the no water treatment. Stem NSC concentration was particularly important for drought
445 resistance and specifically stem soluble-sugars, which were marginally correlated with ΔRGR
446 while starch was not correlated (Table S3). The importance of stem, and specifically soluble-
447 sugars, for drought tolerance suggests that NSCs play a role in maintaining basic functions
448 during prolonged drought. Our data support earlier results (O'Brien *et al.*, 2014), which show
449 that greater NSC concentrations prolong time to death and xylem water potentials both within
450 and among the same species of Bornean trees. We suggest that greater stores of NSC could
451 indicate a larger budget for the dissolution of embolisms, osmoregulation and/or maintenance of
452 cell turgor (Hartmann *et al.*, 2013; Sevanto *et al.*, 2014), and therefore, support the importance of
453 stem NSC in particular for drought resistance.

454 Few NSC variables were significantly correlated with ΔRGR under infrequent-high
455 watering and frequent-low watering. However, a positive correlation between ΔWoody tissue
456 soluble-sugar concentrations and ΔRGR for both treatments was found, which indicates that
457 more resistant species either maintained higher soluble-sugar concentrations by allocating more
458 to woody tissue or that drought resistant species had less demand for soluble-sugars in response
459 to these watering regimes. We hypothesize that adjustments to leaf morphology and below-
460 versus above-ground allocation are more important for maintenance of growth rate in response to
461 mild soil water deficits (Poorter & Markesteijn, 2008; Markesteijn & Poorter, 2009) and that
462 species with greater capacity to express plasticity in these traits place reduced demands on stores
463 of soluble-sugars for drought resistance. Interestingly, ΔWoody soluble-sugar concentration was
464 negatively correlated with baseline total stem NSC, which suggests that low baseline stem
465 storage is related to an inability to move NSC to the stem or high demands for NSC in the stem.

466 *Conclusion*

467 We found a differential response of NSC concentrations in tropical tree seedlings to
468 variability in water availability and severe deficit, which was characterized by an initial decrease
469 in NSC concentration in the early stages of the variable watering regime and an increase in NSC
470 concentration in response to greater water deficit. Furthermore, under severe water deficit high
471 baseline stem NSC concentration expressed a trade-off with fast growth under high water
472 availability and contributed to drought resistance of seedlings. Variability among species in NSC
473 storage under ambient high rainfall conditions may play a mediating role in differential
474 responses of species to the increase in frequency and severity of El Niño driven droughts that are
475 predicted by some climate change scenarios.

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485 methodology and writing. AC contributed to the development of NSC methods and analyzed the
486 NSC. JT helped with the logistics and planning of working in Sabah. AH is scientific PI of the

SBE, wrote the grant application that funded this project and contributed to writing and revisions.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

Methods S1 Details of experimental conditions and trait measurements

Fig. S1 Histogram of rainfall variability over last 25 years.

Fig. S2 Watering patterns of the four treatments.

Fig. S3 Model fits for leaf area estimates from leaf length and width.

Fig. S4 Biomass through time for each species in each treatment.

Fig. S5 Concentration of total NSC, starch and soluble-sugars in leaf, stem and root tissues.

Fig. S6 Morphological and physiological changes in each treatment through time.

Table S1 Compiled information on species distributions.

616 Table S2 Total mortality counts by species and treatment.

617 Tables S3 Correlations between RGR response to treatments and baseline traits.

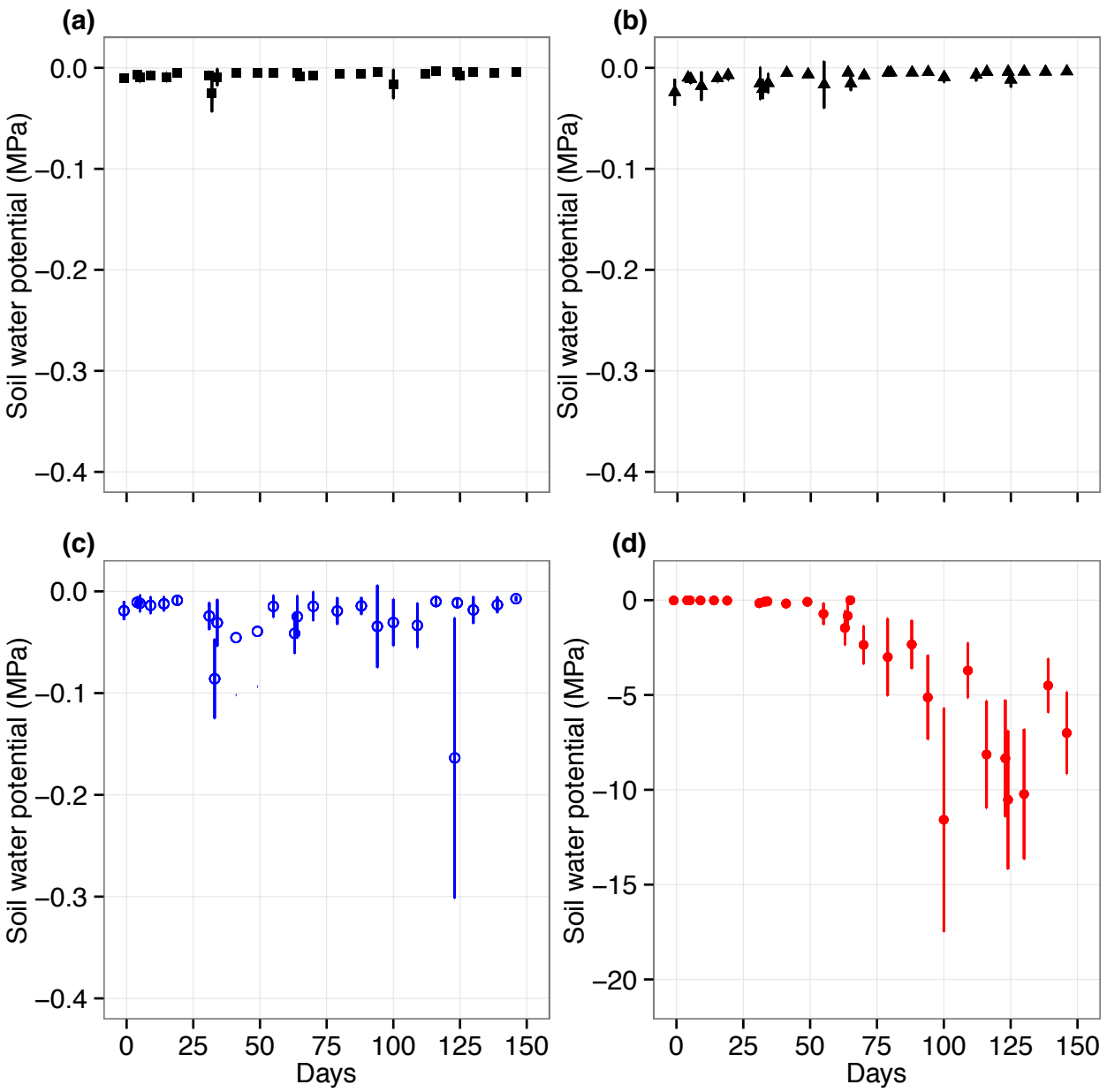
618 Table S4 Correlations between traits.

619 **Table 1.** Species descriptions. Mean allocation metrics (standard error) from the frequent
620 watering treatment (Rooting depth:leaf area – RLA; Specific leaf area – SLA; Shoot:root ratio –
621 SRR; Root mass fraction – RMF; Leaf mass fraction – LMF; Leaf area ratio – LAR).

Species	RLA ($mm\ cm^{-2}$)	SLA ($m^2\ kg^{-1}$)	SRR ($g\ g^{-1}$)	RMF ($g\ g^{-1}$)	LMF ($g\ g^{-1}$)	LAR ($cm^2\ g^{-1}$)
Dryobalanops lanceolata Burck	0.9 (0.2)	20.2 (0.5)	4.2 (0.2)	0.21 (0.01)	0.44 (0.02)	88.8 (4.3)
Durio oxleyanus Griffith	0.5 (0.1)	26.0 (0.5)	3.9 (0.2)	0.22 (0.01)	0.36 (0.01)	93.9 (2.2)
Hopea nervosa King	1.4 (0.1)	22.4 (0.5)	3.5 (0.3)	0.24 (0.01)	0.43 (0.02)	96.4 (5.6)
Koompassia excelsa Taub.	0.7 (0.1)	47.7 (1.1)	4.3 (0.2)	0.20 (0.01)	0.45 (0.01)	214.7 (6.1)
Parashorea malaanonan Merr.	1.6 (0.2)	25.6 (1.3)	3.3 (0.3)	0.26 (0.02)	0.44 (0.01)	114.5 (6.7)
Parashorea tomentella Meijer	0.9 (0.1)	23.7 (1.0)	3.3 (0.2)	0.24 (0.01)	0.45 (0.01)	104.9 (4.4)
Shorea argentifolia Symington	1.6 (0.2)	26.4 (0.5)	4.9 (0.3)	0.18 (0.01)	0.54 (0.01)	144.0 (4.1)
Shorea beccariana Burck	1.2 (0.1)	24.8 (0.7)	4.7 (0.3)	0.19 (0.01)	0.56 (0.01)	137.8 (4.5)
Shorea macrophylla Ashton	0.5 (0.1)	15.7 (0.6)	4.6 (0.2)	0.19 (0.01)	0.42 (0.01)	66.3 (3.7)
Shorea parvifolia Dyer	1.5 (0.2)	25.9 (0.7)	4.9 (0.3)	0.18 (0.01)	0.53 (0.01)	136.8 (4.4)

622
623
624
625

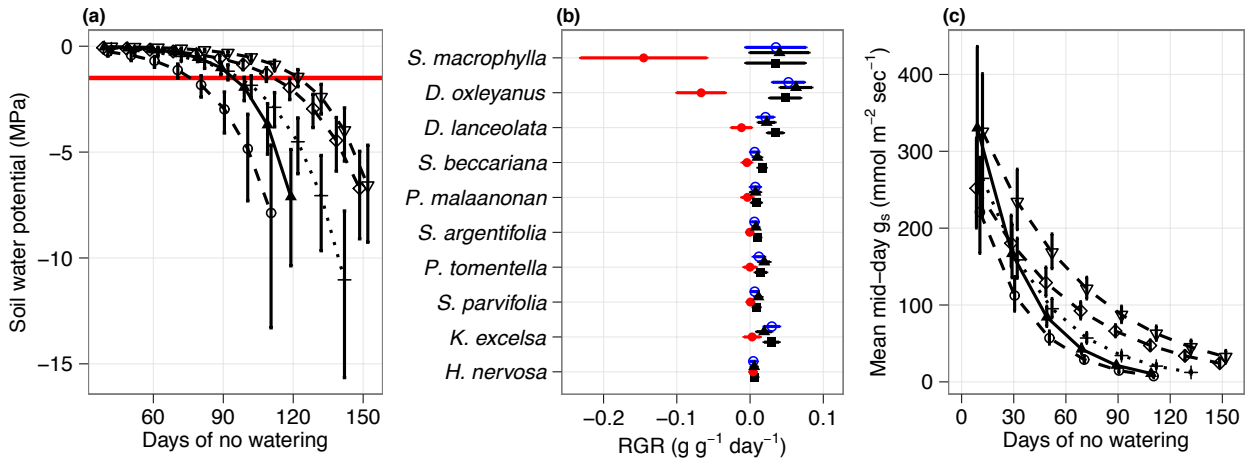
626 **Figures**



627

628 **Fig. 1** The soil water potential (mean ± 95% CI) of each treatment throughout the course of the
629 experiment. (a) control (black-squares), (b) frequent-low watering (black-triangles), (c)
630 infrequent-high watering (blue-circles) and (d) no watering (red-circles). Note the change in
631 scale in panel (d).

632



633

634 **Fig. 2** Soil water potential, relative growth rates (RGR) and stomatal conductance. **(a)** The lines
635 represent the model estimates (s.e.m.) of soil water potential as a function of days of no watering,
636 species and their interaction. Estimates are back-transformed from log-transformed absolute
637 values. The red line represents an assumed wilting point at -1.5 MPa (only *Dryobalanops*
638 *lanceolata*: cross, *Shorea macrophylla*: open circle, *Durio oxleyanus*: closed triangle,
639 *Parashorea malaanonan*: downward triangle, *Hopea nervosa*: open diamond are presented for
640 readability, and they represent the spectrum of initial leaf areas). **(b)** The points represent the
641 mean of each species (95% CI) in each treatment (no water: red-circles, infrequent-high water:
642 blue-circles, frequent-low water: black-triangles and control: black-squares). Species are ordered
643 from the most negative RGR to the least negative RGR in the no watering treatment (top to
644 bottom). All species except *H. nervosa* had significantly slower growth under no watering
645 compared to the control. **(c)** Mean (s.e.m.) mid-day stomatal conductance in the no watering
646 treatment. The same species are presented as in Fig. 2a.

647

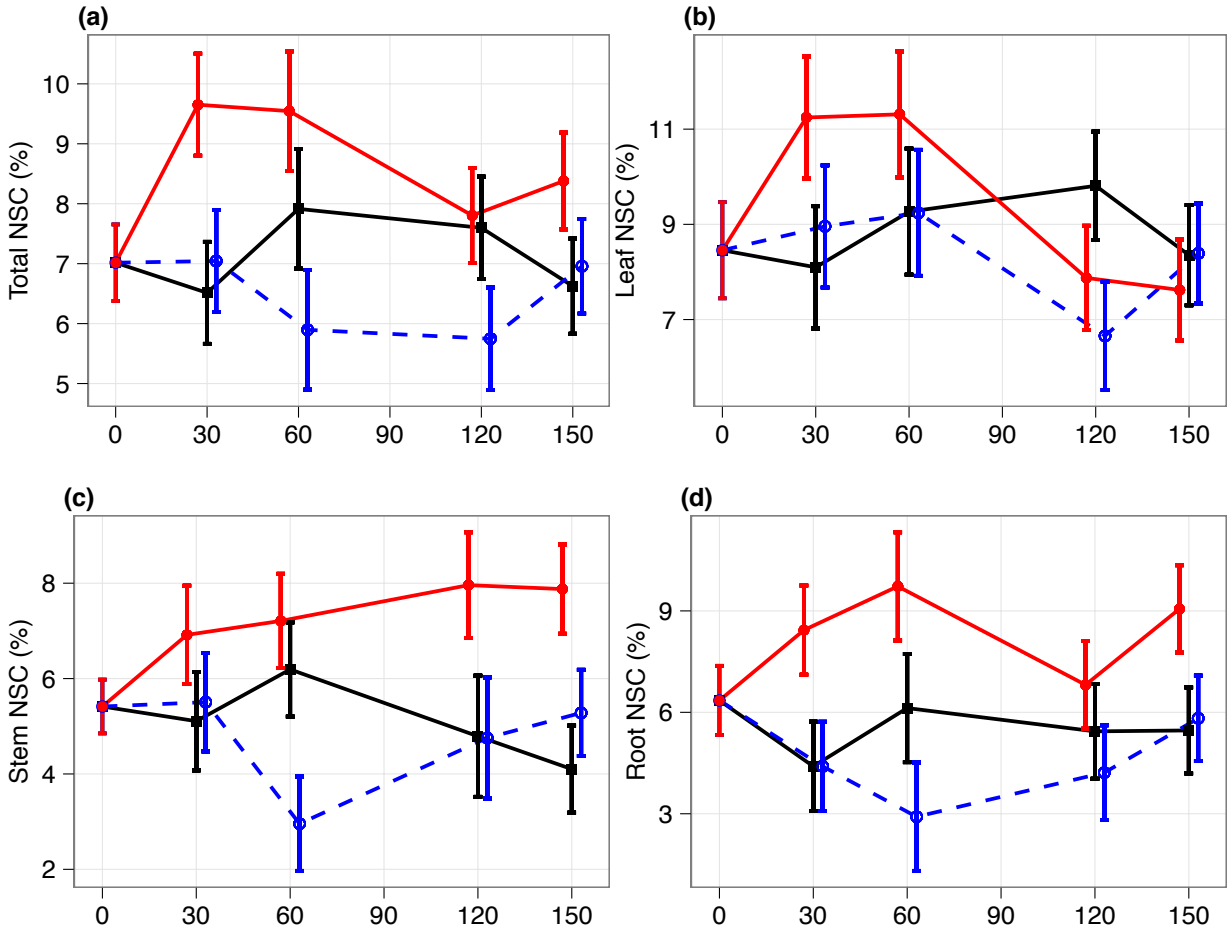


Fig. 3 Change in total, leaf, stem and root non-structural carbohydrates (NSC) for no water (red-circles), infrequent-high water (blue-circles) and the control (black-squares). **(a)** Total NSC was significantly lower at 60 and 120 days under infrequent-high watering and significantly higher at 30 and 150 days under no watering relative to the control. **(b)** Leaf NSC was significantly higher at 30 days under no watering and significantly lower after 120 days under both infrequent-high and no watering. **(c)** Stem NSC was significantly lower at 60 days under infrequent-high watering and significantly higher after 120 days under no watering relative to the control. **(d)** Root NSC was significantly higher after 30 and 150 days under no watering relative to the control. NSC in the controls was statistically indistinguishable from day zero in all tissues at all sampling times. The points represent the mean (s.e.m.) of all species at each time point.

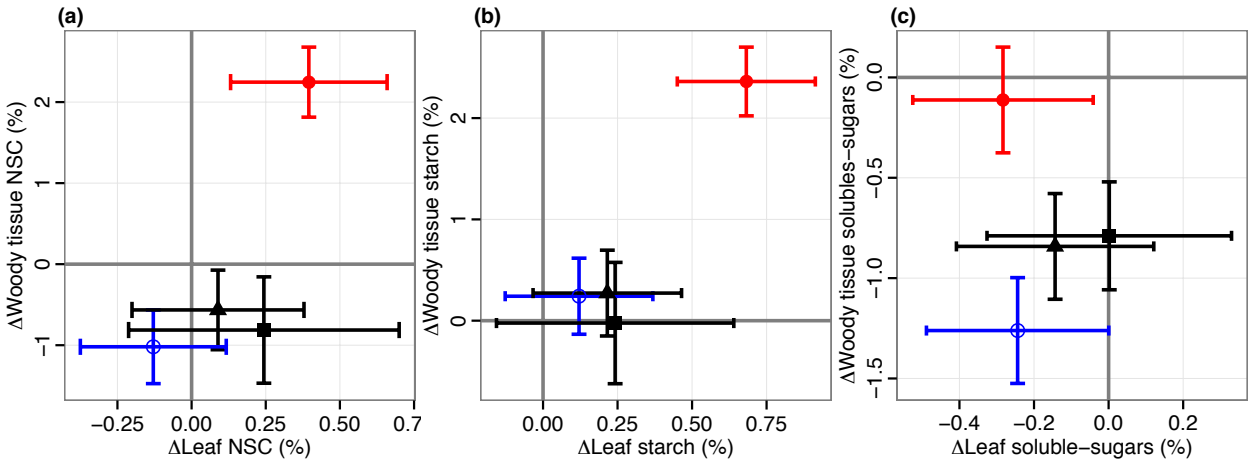
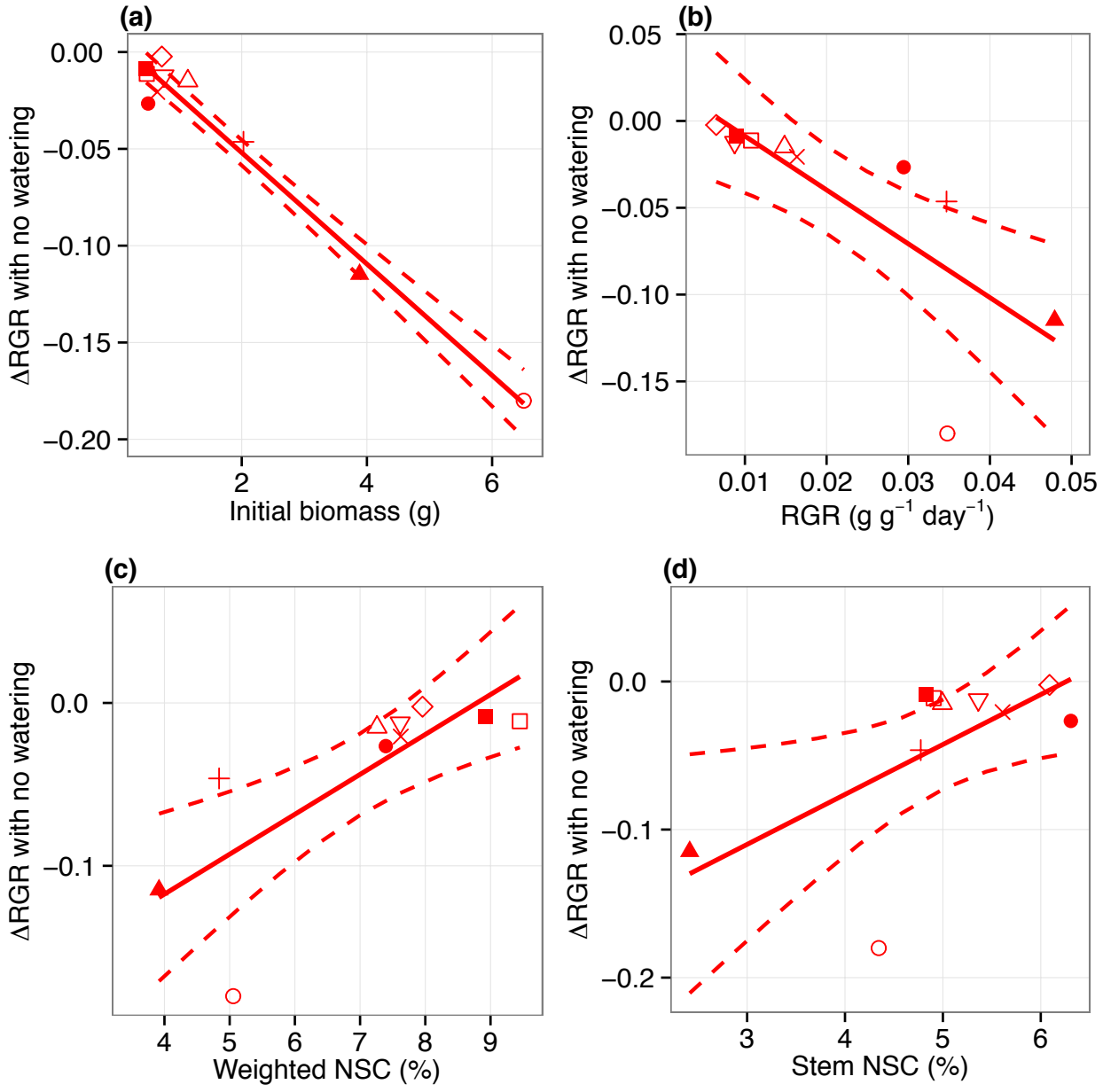


Fig. 4 Change in total NSC, starch and soluble-sugar concentration between initial time-point and all other time-points for all treatments. **(a)** Woody tissue (ΔWoody) had significantly lower NSC concentration under infrequent-high watering (blue-circle) and significantly higher NSC under no watering (red-circle) while NSC concentration in leaf tissue (ΔLeaf) was not statistically different from the initial time-point. Control (black-squares) and frequent-low (black-triangles) watering were statistically similar in both tissues through time. **(b)** Starch concentration in woody tissue was significantly higher through time under no watering but was similar through time for all other treatments. Starch in leaf tissue was statistically indistinguishable among treatments and through time. **(c)** Only no watering had a similar soluble-sugar concentration through time in woody tissue while all other treatments had significant decreases in woody tissue soluble-sugars with infrequent-high watering declining the most through time. Leaf tissue soluble-sugar was statistically indistinguishable among treatments and through time.

675



676

677 **Fig. 5** The difference in RGR between a treatment and the control watering treatment (ΔRGR)

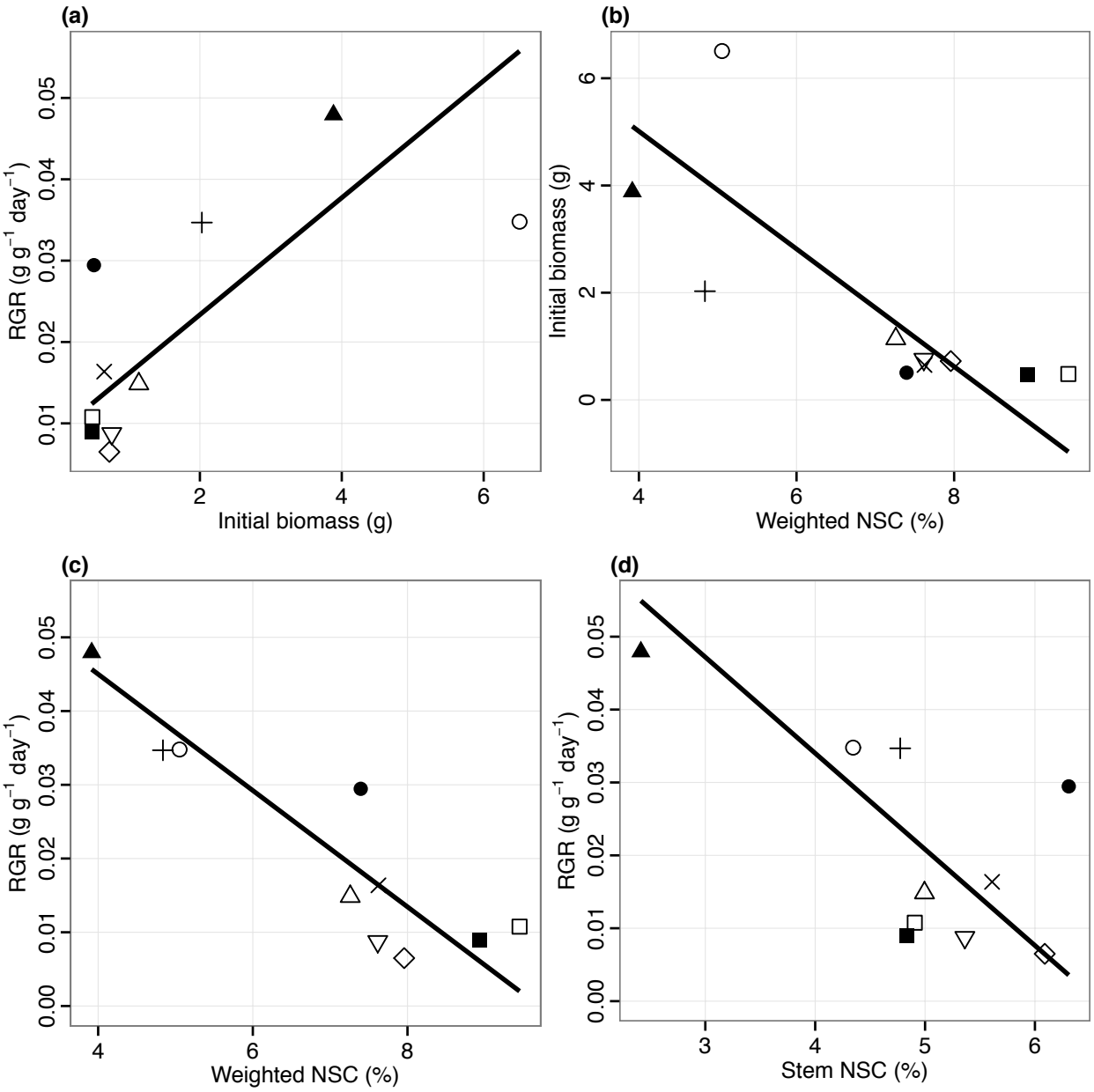
678 as a function of baseline traits. **(a)** ΔRGR declined significantly with increasing initial seedling

679 biomass. **(b)** ΔRGR declined significantly with increasing RGR in the control treatment. **(c)**

680 ΔRGR increased significantly with increasing baseline total NSC. **(d)** ΔRGR increased

681 significantly with increasing baseline stem NSC. Lines are generalized least squares model fits,
682 and dashed-lines represent 95% CIs. Points represent mean values for each species
683 (*Dryobalanops lanceolata*: cross, *Durio oxleyanus*: closed triangle, *Hopea nervosa*: open
684 diamond, *Koompassia excelsa*: closed circle, *Parashorea malaanonan*: downward triangle, *P.*
685 *tomentella*: upward triangle, *Shorea argentifolia*: open square, *S. beccariana*: x, *S. macrophylla*:
686 open circle, *S. parvifolia*: closed square).

687



688

689 **Fig. 6** Relationships between mean baseline trait values among species. **(a)** Relative growth rate
690 (RGR) was significantly positively correlated with initial biomass, but both **(b)** biomass and **(c)**
691 RGR traded off with baseline non-structural carbohydrate (NSC) concentration. **(d)** RGR also
692 traded off with baseline stem NSC. Standardized major-axis lines are used for visualization of
693 the relationship, and points represent mean values for each species (*Dryobalanops lanceolata*:
694 cross, *Durio oxleyanus*: closed triangle, *Hopea nervosa*: open diamond, *Koompassia excelsa*:

closed circle, *Parashorea malaanonan*: downward triangle, *P. tomentella*: upward triangle, *Shorea argentifolia*: open square, *S. beccariana*: x, *S. macrophylla*: open circle, *S. parvifolia*: closed square).

Supporting Information Methods S1, Fig. S1-6, Tables S1-4

Methods S1 Details of experimental conditions and trait measurements

Shadehouse details

The shadehouses provided the seedlings with an average (SE) of 3.9% (0.1) light and a red:far-red ratio (SE) of 1.07 (0.01). Light was measured by simultaneous shadehouse and open sky photosynthetically active radiation sensors (SKP 210 quantum sensor; Skye instruments LTD, Llandrindod Wells, Powys, UK) logged every 10 minutes for a 24-hour period in each shadehouse.

The average (SE) daily temperature during the course of the experiment was 25.4 C (0.06) with average maximum and minimum temperature of 30.9 C and 22.7 C, respectively. The average (SE) daily humidity was 92.5% (0.19) with average maximum and minimum humidity of 98.9% and 71.2%, respectively. Temperature within the shadehouses was on average 0.22 C (95% CI: 0.16 – 0.29) hotter but humidity was not significantly higher than values measured in direct light at the Sabah Biodiversity Experiment. Temperature was measured with Thermocron Ibuttons (model DS1921G-F5#, Maxim Integrated Products, Inc., Sunnyvale, CA, USA) at 3 hour intervals throughout the experiment, and humidity was measured using a relative humidity probe at 30 minute intervals (SKH 2000 probe, Skye instruments LTD, Llandrindod Wells, Powys, UK).

Seed details

Seeds from ten species of shade-tolerant forest trees were collected during a landscape scale masting event in August 2010. We collected seeds directly from three different mature trees for

each species except for *Durio oxleyanus* and *Hopea nervosa* seeds, which were obtained from only one mature tree. Seeds were not planted on the same day because seed maturation rates varied among species, but all seeds were planted between the 10th and 29th of August. We weighed each seed and placed them under wet hessian sacks to germinate. After germination, we planted 33-34 seeds from each mature tree (100 for *Durio oxleyanus* and *Hopea nervosa* as there was only one mature tree for each) for a total of 100 seeds per species.

Soil details

Pots were filled with homogenized forest soil obtained from the Innoprise-FACE Foundation Rainforest Rehabilitation Project (www.face-thefuture.com). The soil used was classified as clay and comprised 50% clay, 30% silt and 20% sand, which is similar to mid-slope sites at the Malua Forest Reserve.

Calculation of soil water potential

A range of volumetric soil moisture from 1.5% to 46.0% was used to develop two linear equations between volumetric soil moisture and soil water potential. Two equations were required because soil water potential declined at a faster rate below 28% volumetric soil moisture (O'Brien *et al.*, 2013).

Vapour Pressure Deficit

We measured temperature and humidity every thirty minutes throughout the course of the experiment, which we used to calculate vapor pressure deficit (Murray, 1967; Monteith & Unsworth, 1990).

Additional trait measurements

In order to examine leaf senescence, we measured chlorophyll content on one leaf from the top, middle and bottom part of the canopy of each seedling using a spadmeter (model Spad-502, Konica Minolta Sensing, Inc., Osaka, Japan).

Trait response

Seedlings had less biomass after long-term water deficit in the no-watering treatment relative to the frequent-high watering treatment (difference in biomass between frequent-high watering and no-watering after 4 months = -2.1 g, -3.2 – -1.0; Fig. S6a). SLA declined similarly for all treatments after 150 days (difference in SLA between month zero and five = -6.7 m^2 kg^{-1}, -9.1 – -4.3; Fig. S6b). Daily stomatal conductance (g_s) declined in the no-watering treatment at a rate of -2 mmol m^2 sec^{-1} per day (-2.4 – -1.5; Fig. S6c). Allocation to roots was not substantially altered by drought treatments as RMF never differed significantly from the frequent-high watering for any drought treatment, and RLA was only significantly higher than frequent-high watering in the no-watering treatment after 4 months (difference in RLA between no watering and frequent-high watering = 1.1, 0.3 – 2.2; Fig. S6d). However, LMF was significantly lower in the no-watering after only 4 months due to leaf loss (difference in LMF between no-watering and frequent-high watering = -0.05, -0.1 – -0.02; Fig. S6e). In the no watering treatment, pre-dawn leaf (Ψ_l) and xylem (Ψ_x) water potentials were significantly lower after 2 months and decreased to values as much as -1.0 MPa lower than the other treatments (difference in Ψ_l and Ψ_x between no-water and other treatments = -0.8 MPa, 95% CI: -1.1 – -0.6 and -1.0 MPa, -1.3 – -0.8, respectively), which implies that night-time rehydration was inhibited.

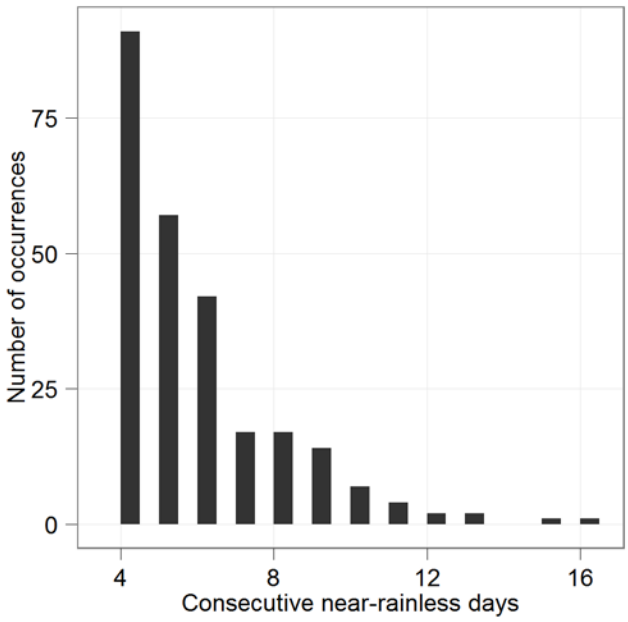


Fig. S1 Histogram of rainfall variability over the last 20 years from daily rainfall data collected at the Danum Valley Field Centre.

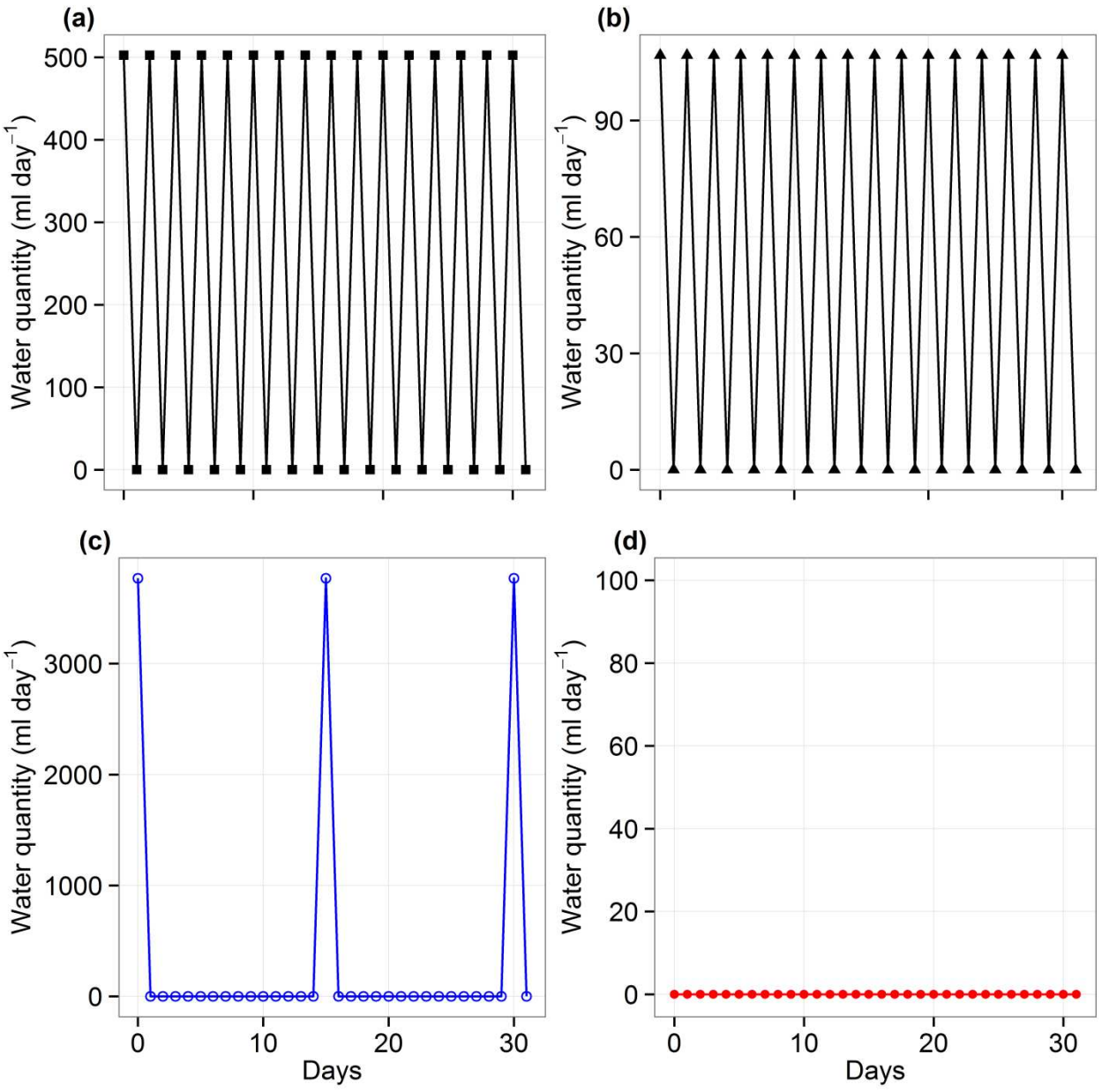


Fig. S2 Example of watering patterns for the four treatments over a 30 day period: **(a)** frequent-high water (control), **(b)** frequent-low water, **(c)** infrequent-high water and **(d)** no water.

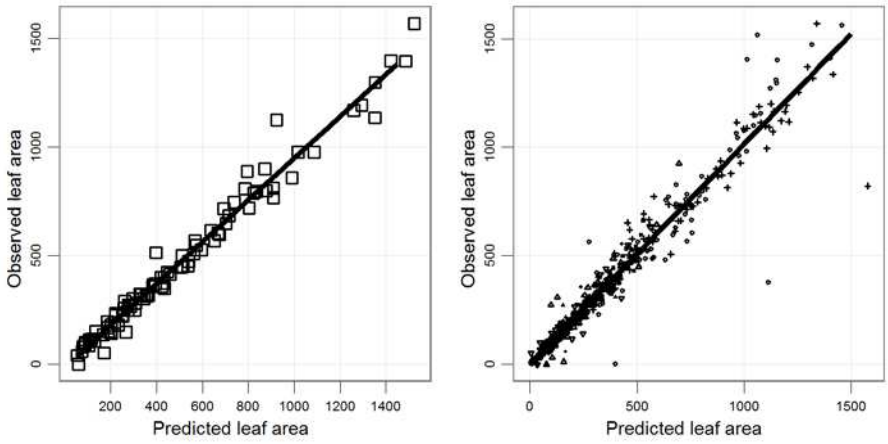


Fig. S3 Model fits of leaf area for *Koompassia excelsa* (left panel) and all other species (right panel). Because *K. excelsa* had a composite leaf with many leaflets, the equation *la* = *length*width* was used to estimate individual leaflet area and then scaled up to total leaf area by multiplying mean leaflet area by the number of leaflets. Total plant leaf area was the sum of all leafs (R^2 = 0.98, n = 89). The leaf area of all other species was calculated with the equation *la* = *species* + *length* + *width* + *length*width*. Total plant leaf area was the sum of all leaves for an individual plant (R^2 = 0.95, n = 783). Outliers were due to poor estimates of dieback.

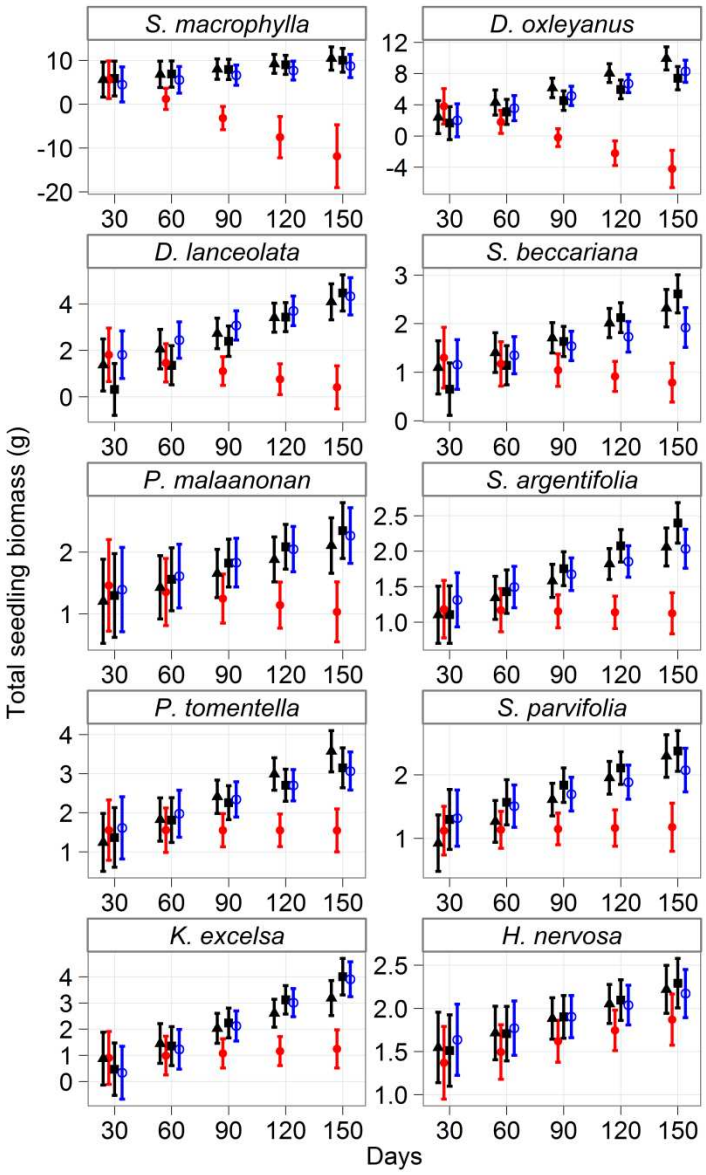
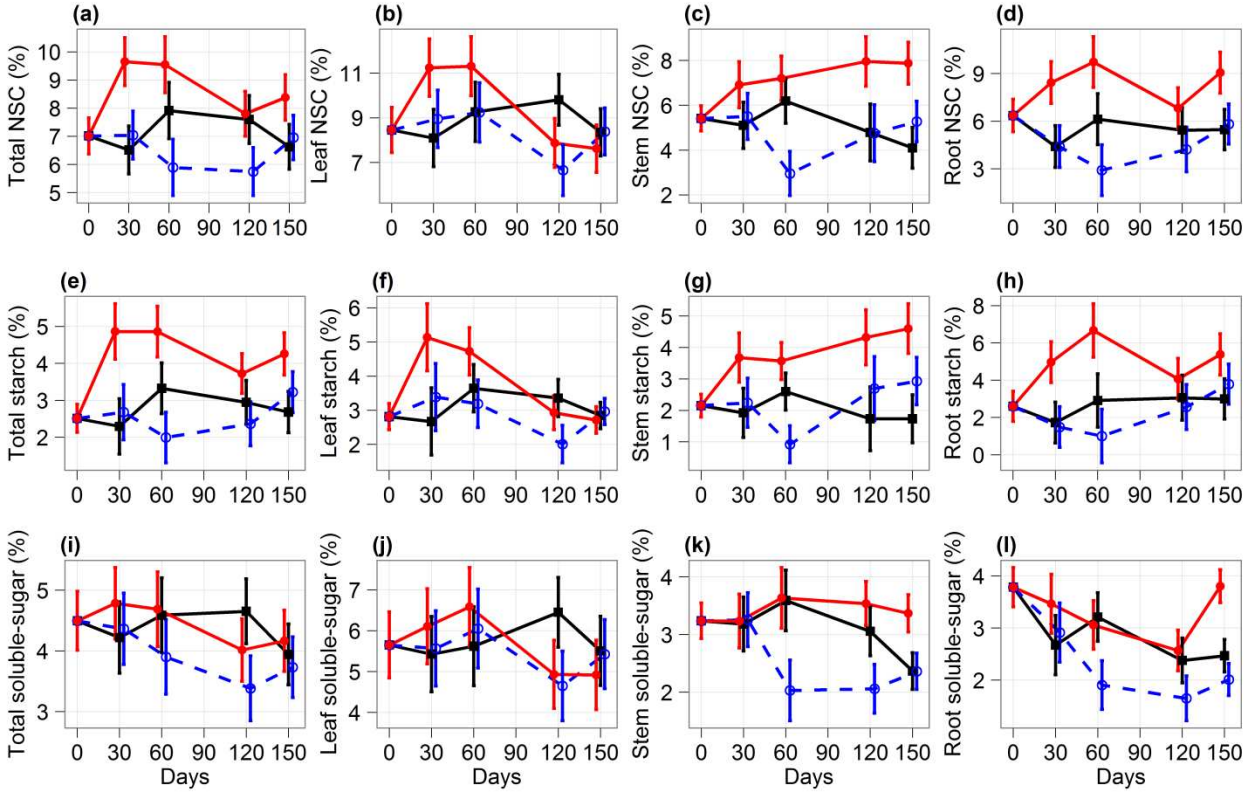
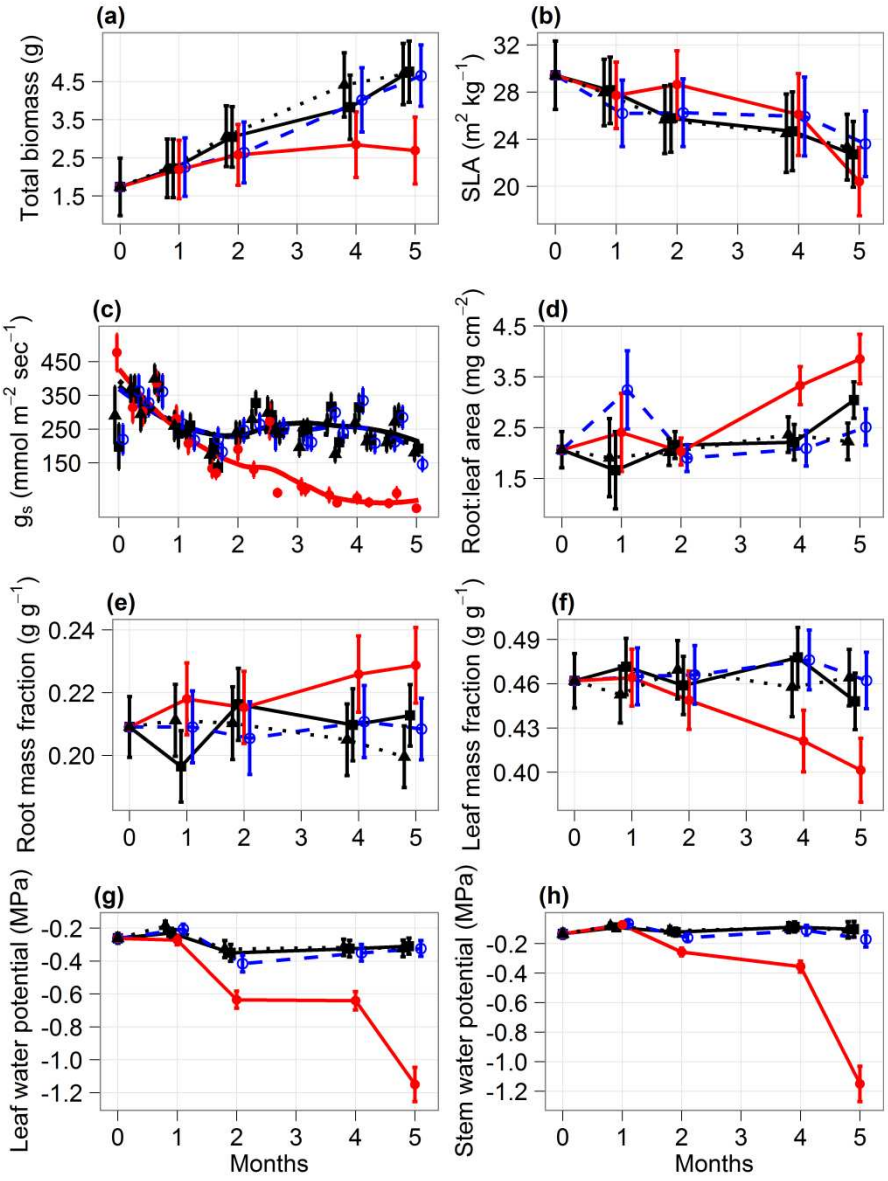


Fig. S4 Biomass response to drought as a function of days since the start of the experiment. The points represent the mean of each species (95% CI) at each time point (no water: red circle, infrequent: blue open circle, frequent-high: black square and frequent-low: black triangle). Species are ordered in terms of greatest to least leaf area response to the no watering treatment (top left to bottom right). Full species names are *Dryobalanops lanceolata*, *Durio oxleyanus*, *Hopea nervosa*, *Koompassia excelsa*, *Parashorea malaanonan*, *P. tomentella*, *Shorea argentifolia*, *S. beccariana*, *S. macrophylla*, *S. parvifolia*.



85

86 **Fig. S5** Change in NSC concentrations through time for control (black line and squares),
87 infrequent-high (blue-dashed line and open circles) and no watering treatments (red line and
88 closed circles). **(a)** Total NSC weighted by organ biomass and **(b-d)** NSC concentration in each
89 organ. **(e)** Total starch weighted by organ biomass and **(f-h)** starch concentration in each organ.
90 **(i)** Total soluble-sugar weighted by organ biomass and **(j-l)** soluble-sugar concentration in each
91 organ. The points represent the mean across species (s.e.m.) at each time point. The frequent-
92 low watering treatment was statistically indistinguishable from the frequent-high watering
93 treatment and was therefore left off the figure to improve readability.



94

95 **Fig. S6** Trait response to different watering treatments through time. Points represent means

96 (95% CI) across all species in each treatment (red = no watering, blue = infrequent-high

97 watering, black square = control watering and black triangle = frequent-low watering treatment).

98 Non-overlapping confidence intervals represent significant differences.

Table S1 Current knowledge of species distributions in lowland Borneo rainforest for the ten species used in this experiment. Altitude and site preferences were compiled from multiple sources. (Wood & Meijer, 1964; Lemmens *et al.*, 1995; Soepadmo & Wong, 1995).

Species	Altitude	Site preference
Dryobalanops lanceolata Burck	below 600 m	prefers alluvial over ultrabasic and deep moist soils
Durio oxleyanus Griffith.	below 400 m	frequently flooded clay-rich alluvium
Hopea nervosa King	below 600 m	flat to slightly undulating, deep clay soils
Koompassia excelsa Taub.	below 600 m	stream valleys and lower slopes
Parashorea malaanonan Merr.	below 1300 m	water inundated to dry and rocky; absent from sandy alluvium
Parashorea tomentalla Meijer	below 200 m	handles occasional flooding, clayey soil
Shorea argentifolia Symington	below 900 m	well drained soils, alluvium, slope to ridges, susceptible to flooding
Shorea beccariana Burck	below 1300 m	dry ridge-tops of sandstone, sandy clayey soils
Shorea macrophylla Ashton	below 600 m	riparian, well-watered near stream
Shorea parvifolia Dyer	below 750 m	most common species on well drained soils, yellow clay, susceptible to flooding

Table S2 Total mortality counts by species and treatment.

Species	Freq-High	Freq-Low	Infrequent	No-Water
D. lanceolata	1	0	0	9
D. oxleyanus	0	0	0	13
H. nervosa	0	0	0	2
K. excelsa	0	1	1	3
P. malaanonan	0	0	0	6
P. tomentella	0	0	0	3
S. argentifolia	1	0	0	5
S. beccariana	2	1	1	6
S. macrophylla	1	0	1	12
S. parvifolia	0	3	0	6

Table S3 Pearson correlation coefficients between mean trait values for species from frequent-high watering treatment and the difference in RGR between the frequent-high watering and drought treatments. Non-structural carbohydrate values are weighted by tissue mass for total plant values. Ψ_x- Ψ_s represents the difference between xylem water potential and soil water potential at the last two harvests. ΔStem NSC represents the difference in intial NSC concentration and final NSC concentration in the stem. Bold values are p-values < 0.05.

	ΔRGR to no watering	ΔRGR to infrequent watering	ΔRGR to frequent-low watering
RGR (cm^2 cm^{-2} day^{-1})	**-0.76**	0.21	0.25
Ψ_x - Ψ_s	-0.05	0.03	0.22
Initial size (g)	**-0.98**	0.34	0.51
Weighted NSC	**0.77**	-0.14	-0.31
Total starch	0.58	0.13	-0.35
Total soluble-sugars	0.57	-0.24	-0.16
Total leaf NSC	0.52	-0.04	-0.09
Total stem NSC	**0.63**	-0.34	**-0.75**
Total root NSC	0.36	0.12	-0.13
Stem starch	0.24	0.03	-0.59
Stem soluble-sugars	0.60	-0.47	-0.45
ΔStem soluble-sugars no water	0.20	NA	NA
ΔStem soluble-sugars frequent-low	NA	NA	**0.79**
ΔStem soluble-sugars infrequent-high	NA	**0.74**	NA

Table S4 Pearson correlation coefficients between mean traits. Except for changes in stem NSC, all traits were calculated from seedlings harvested in the frequent-high watering treatment. Bold values are p-values < 0.05.

	RGR	$\Psi_x - \Psi_s$	Size	NSC	Starch	Soluble sugars	Leaf NSC	Stem NSC	Root NSC	Stem starch	Stem soluble-sugars
$\Psi_x - \Psi_s$	0.38										
Initial size	**0.71**	0.07									
Weighted NSC	**-0.90**	-0.34	**-0.77**								
Total starch	-0.53	-0.33	-0.59	0.55							
Soluble-sugars	**-0.75**	-0.21	-0.56	**0.86**	0.05						
Leaf NSC	**-0.71**	-0.31	-0.54	**0.89**	0.24	**0.92**					
Stem NSC	**-0.65**	-0.29	**-0.63**	**0.64**	**0.67**	0.36	0.33				
Root NSC	-0.31	-0.04	-0.31	0.06	**0.65**	-0.32	-0.36	0.43			
Stem starch	0.01	-0.11	-0.29	0.07	**0.72**	-0.36	-0.20	**0.63**	0.50		
Stem soluble-sugars	**-0.85**	-0.28	-0.55	**0.77**	0.22	**0.78**	0.60	**0.73**	0.11	-0.07	
ΔWoody soluble-sugars no watering	-0.04	0.37	-0.21	0.11	**0.66**	-0.28	-0.16	0.43	0.58	**0.72**	-0.08
ΔWoody soluble-sugars frequent-low	0.23	0.09	0.53	-0.17	-0.24	-0.06	0.04	**-0.66**	-0.21	-0.47	-0.43
ΔWoody soluble-sugars infrequent-high	0.50	0.08	0.46	-0.38	-0.36	-0.23	-0.12	**-0.71**	-0.28	-0.29	**-0.66**

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